Guidelines for Protocols Involving Clinical Assessment of Antimalarial Drug Resistance (clinical evaluation)

The present guidelines for protocols involving clinical assessment of antimalarial drug resistance (*in vivo* test) are based on the recommendations by The WHO unit on Anti-infective Drug Resistance Surveillance and Containment (DRS) for assessing the *in vivo* response. The criteria take into account the traditional WHO 28 day *in vivo* test (WHO 1973), the WHO 1996 criteria (WHO 1996), and the views of malariologists. The criteria involve *(i) clinical criteria for study entry and withdrawal, (ii) haematological criteria and (iii) parasite counts during follow-up,*

Study Population

The study population will consist of male and female patients with acute uncomplicated falciparum malaria aged less than 5 years who consent to participate in the studies.

Sample size determination

Three hundred patients will be enrolled at each of the participating sites over an initial period of 2 years. This should allow each participating center to have enough samples to determine any associations between parasite gene mutations and drug resistance, with an odds ratio of 2.5 or more at the 95% confidence level and power of 80% (EPI Info, CDC, Atlanta). Details of the sample size calculations are provided in the attached standard operating procedures developed for the Network.

Entry criteria

Criteria for inclusion

Criteria for patient inclusion should include:

- Age between 6 and 59 months (i.e., under 5 years).
- Signs/symptoms of acute uncomplicated falciparum malaria (WHO 1987).
- Mono-infection with *P. falciparum*, with asexual blood stage parasitaemia in the range of 2,000 to 100,000 asexual parasites per μL.
- Willingness to give consent to participate.

- Absence of severe illness.
- Presence of axillary temperature of ≥37.5 °C but < 39.5 °C

Criteria for exclusion

- Presence of signs of severe complicated falciparum malaria (WHO, 1989).
- *Cerebral malaria (unrousable coma)*
- *Vomiting > twice within preceding 24 hours*
- *One convulsion within preceding 24 hours*
- *Inability to drink or breast-feed, or to take oral medication.*
- *Pulmonary oedema*
- *Circulatory collapse/shock*
- *Spontaneous bleeding/disseminated intravascular coagulopathy (DIC)*
- *Macroscopic haemoglobinuria (urine red or coca cola colour)*
- *Jaundice*
- *Other manifestations of complicated malaria (hyperparasitaemia: >5% RBC infected, hyperpyrexia (axillary temperature > 39.5 °C).*
- *A clear history of adequate treatment with drugs expected to be effective in the study area in the preceding 72 hours.
- History of taking drugs with antihistaminic actions, i.e., chlorpheniramine, promethazine, chlorpromazine) within the past 2 days at sites where chloroquine is the first line antimalarial drug.
- Presence of underlying diseases (cardiac, renal, hepatic diseases, malnutrition, gastrointestinal diseases).
- History of allergy to study drug.
- Inability to come for the stipulated follow-up visits, or difficulty in accessing the health facility, or any situation or condition which may compromise ability to comply with the trial procedures.
- **Severe normocytic anaemia (haemoglobin < 5 g/dL, or haematocrit < 15%)*
- **Hypoglycaemia (< 40 mg/dL)*

**Note: The detection of antimalarial drugs on Day 0 prior to drug administration (baseline) by drug determination in whole blood (HPLC) at the later phase will be used at data analysis phase.*

Study procedures

Pre-treatment assessments

Prior to antimalarial treatment, the following assessments should be performed:

- Clinical assessments: Physical examination and monitoring of vital signs symptoms and body temperature (axillary temperature: ^{o}C).
- Finger prick blood sample for microscopic (malaria parasitaemia) examination
- Finger prick blood sample (100 ul heparinized cap. Tubes spotted on well absorbing filter paper (Whatman 3). for baseline concentrations of chloroquine (and / or its active metabolite --*n*-desethylchloroquine), amodiaquine (and / or its active metabolite –*n*-diethylamodiaquine).
- Finger prick blood sample for molecular markers on filter paper (following detailed protocol in section IV on molecular assays).
- Venous blood sample for full blood count and chemistry (anticoagulant) (1.0 ml each)
- Venous blood sample for determination of sulphadoxine, pyrimethamine concentrations (0.8 mls). (detailed protocol in section II on determination of drug levels in blood for details)
- Venous blood sample for *in vitro* sensitivity test and parasites culture (1.0 ml in CPD anticoagulant

Special attention should be paid to storage conditions of samples to prevent deterioration. All sites should strictly implement the storage protocols described in the guidelines. Provision should be made to deposit duplicate samples in a common repository as agreed by the network.

Antimalarial Treatment

- Treatment doses should be based on the patient's body weight.
- All treatment doses should be given under supervision, and the patient will be observed for at least 1 hour post administration to ascertain retention of the drug. If the patient vomits within the first 30 minutes post administration, the treatment should be repeated with the same dose. If the patient vomits between 30 minutes and 1 hour after dosing, half dose will be administered. Children with persistent vomiting should be excluded from the study and

referred urgently to the appropriate health facility after giving the first dose of the parenteral drug of choice for the sites. These data must be recorded in the *Patient Record File (PRF)*.

- ***Antimalarial drug formulation and dose strength***
  - Chloroquine will be dispensed orally as 150 mg base tablets.
  - Amodiaquine will be dispensed orally as 200 mg base tablets.
  - Sulphadoxine /pyrimethamine will be dispensed orally as tablets (500 mg sulphadoxine, 25 mg pyrimethamine).
  - Drugs will be obtained centrally from reputable pharmaceutical companies and distributed to the study sites.

The study medication should be stored at room temperature in cool dry lock up cupboards protected from light.

- ***Recommended treatment regimens:***
  - ***Treatment with chloroquine, amodiaquine:***

    3-day course with the following doses:
    - Day 0: 10 mg base per kg body weight
    - Day 1: 10 mg base per kg body weight
    - Day 2: 5 mg base per kg body weight
    - Fractions of the tablets will be rounded up to the nearest quarter.
  - ***Treatment with sulphadoxine / pyrimethamine (SP):***
    - SP will be given as a single dose equivalent to *25 mg/kg body weight based on sulphadoxine component, given once daily).*

Concomitant medication

- In patient receiving CQ as 1st line, treatment with any drugs with antihistaminic action including all antiemetics, e.g., chlorpheniramine, promethazine, chlorpromazine, etc., is contraindicated during the trial period.
- Folate should not be given to patients receiving sulphadoxine / pyrimethamine at any time during the follow-up.
- Iron may be given at the discretion of the treating clinician, following local recommendations, if they exist.

- Blood transfusions can be given as *per* local recommendations. Patients requiring blood transfusions will be withdrawn from the study.
- All other adjunct treatments may be administered as required during the trial period. For example, the administration of analgesics such as paracetamol on day 0, 1 and 2 is permissible if the patient's condition warrants such medication, however drugs that may not be administered during the trial period include tetracycline, quinoline antibiotics, chloramphenicol, co trimoxazole and rifampicin.
- Any use of concomitant medications should be recorded in patient's record chart in details (dose, date and time given and stopped, generic/trade name, etc.).

Alternative treatment of drug failures

- Any patients who fail treatment with the trial medication will be treated with an effective alternative antimalarial drug in keeping with the local policy of that area. For example, the recommended alternative antimalarial treatment will be sulphadoxine / pyrimethamine when the drug under test is chloroquine or amodiaquine, and quinine or mefloquine in the case of poor response to sulphadoxine / pyrimethamine.

    - ***Treatment with sulphadoxine / pyrimethamine:***
        - Sulphadoxine / pyrimethamine will be given as a single dose equivalent to.25 mg p sulphadoxine per kg body weight (up to a maximum adult dose of 3 tablets).
    - ***Treatment with mefloquine:***
        - Mefloquine will be given as a single dose of 15 mg base per kg body weight.
    - ***Treatment with quinine:***
        - Quinine will be given as oral doses of 10 mg (sulfate) per kg body weight every 8 hours for 7 days.

- If the patient develops any signs of severe or complicated malaria or any of the general danger signs during the follow-up period, he or she should be given the first dose of the rescue drug specific to the site and taken urgently to the appropriate health facility.

? Once a patient is treated as a treatment failure, he/she will be discontinued from the study. All protocol investigations up to and including the day of treatment failure will be done. Thereafter, the patient will not have other protocol investigations done. The investigator is obliged to follow up the patient till cure. These groups of patients will be assigned to another cohort and analysed for the 4 parameters being studied by the network.

Assessments during the studies

Follow-up period: Follow-up period of 28 days is recommended for all sites. The parent/guardian should be instructed to bring the child to the clinic/health centre at any time during the follow up, if the child is still sick or if there is any cause of worry.

Clinical assessments

Clinical assessments during the study will include physical examination and monitoring of vital signs done on days 0, 1, 3, 5, 7, and 14. Patient treated with SP will be followed up on days 21 and 28 in addition.

- *Body temperature:* Axillary temperature should be recorded in PRF with one decimal point, preferably with electronic thermometers. If the axillary temperature is less than 36.0 ^{O}C, the measurement must be repeated.

Microscopic blood examination

- Patient's peripheral blood parasitaemia (asexual and sexual form) should be assessed once daily on days 0, 1, 3, 5, 7 and 14. Patient treated with SP will be followed up on days 21 and 28 in addition. Parasite count should be recorded in PRF.
- For each assessment, 2 slides should be prepared, i.e., one slide with thick film for screening, and one slide with thick and thin film for calculation of parasite density and speciation. Preparation and staining of the blood slides will follow the procedures outlined in *Basic Malaria microscopy, Part 1* (WHO 1991). Slides will be stained with Giemsa stain at pH 7.2. The number of asexual parasites will be counted against at least 200 white blood cells (WBC).
- *Malaria parasite count:* At screening, prior to enrolment, 20 high power fields will be examined on the thick film. Adequate parasitaemia for enrolment requires at least 1 parasite for every 5 WBC, corresponding to approximately 2,000 asexual parasites per ?L. A second Giemsa stained thick film will be examined to quantify the parasitaemia. Parasitaemia is measured by counting the number of asexual parasites against a number of WBC in the thick blood film, based on a putative count of 8,000 WBC per ?L as adequate mean WBC in the population under investigation. The number of asexual parasites is counted against at least 200 WBC (enumeration on the field being counted when reaching 200 WBC must be completed). The parasitaemia (in ?L) is calculated using the formula:

$$\text{Parasitaemia } (/? \text{L}) = \frac{\text{Number of parasites x WBC count (8,000)}}{\text{Number of WBC counted (200)}}$$

If > 500 parasites have been counted without having reached 200 WBC, the count is stopped after completion of count in the last field. The parasitaemia is calculated according to formula above. If *P. falciparum* gametocytes are seen, a gametocyte count is performed against 1,000 WBC.

Haematological assessments

- Quantitative determination of haematocrit and/or haemoglobin level should be performed on days 0, 7 and 28 and whenever there is clinical failure. Haematocrit can be measured by means of the micro-haematocrit method described by Levy and Lambert (1974).
- Full blood count and chemistry should be performed on days 0, 7 and 28 and whenever there is clinical failure.

Assessments of molecular markers

- Finger prick blood for molecular markers will be collected on days 0, 7, 14, 21 and 28 following detailed protocol in section on assessment of molecular markers of resistance Part-IV).

Determination of antimalarial drug concentrations

- Filter paper blood samples will be collected for determination of whole blood concentration of (chloroquine, amodiaquine mefloquine, halofantrine or artemisinine derivatives). Venous blood samples will be collected for the determination of whole blood concentrations of sulfadoxine, pyrimethamine). Blood (0.5 mL) will be collected from all patients following the initiation of treatment on days 0, 1, 3, 7, 14, 21, 28 and during recrudescence following detailed protocol in section on assessment of antimalarial drug levels PART -III.

Assessments at time of parasite recurrence

Assessments at the time of parasite recurrence should include:

- Clinical assessments: physical examination, monitoring of vital signs resting respiratory rate and pulse (before obtaining finger prick sample for blood film) & symptoms, and body temperature (axillary, ^{o}C)
- Finger prick blood for microscopic examination and determination of parasite density.
- Finger prick blood for determination of haemoglobin / haematocrit.
- Finger prick blood for molecular markers.
- Venous or finger prick blood for antimalarial drug levels (venous / finger prick).
- Venous blood for *in vitro* sensitivity test.

? Venous blood for haematology and blood chemistry.

Criteria for withdrawal of patient from the study

? Danger signs / symptoms of severe malaria as per the WHO definition (WHO, 1990), or a clinical requirement for parenteral therapy.

? Any serious adverse events or severe adverse events requiring withdrawal from the study

? Vomiting after repeated doses.

? Need to use medicaments with antimalarial activity, for the treatment of infections other than malaria during study period (e.g., tetracycline, doxycycline, cotrimoxazole) during treatment with antimalarial.

? Need to use drugs with antihistaminic activity, i.e., chlorpheniramine, promethazine, chlorpromazine, etc., during study period.

? Withdrawal of consent by patient/parent/guardian.

? If the patient is lost to follow up

Criteria for discontinuation

If at any time the progress of the child is unsatisfactory, the child's parent/guardians will be instructed to bring the child to the clinic/health centre. Objective criteria for discontinuation include:

? Danger signs/symptoms of severe malaria as per the WHO definition (WHO 1990), or a clinical requirement for parenteral therapy.

? Day 3 Parasite count $\geq$ 25% of day 0 count.

? Parasitaemia present on day 7

? Recurrent parasitaemia within 28 days of enrollment.

? Need to use medicaments with antimalarial activity for the treatment of infections other than malaria (e.g., tetracycline, doxycycline, cotrimoxazole) during treatment with antimalarial.

? Need to use drugs with antihistaminic activity, i.e., chlorpheniramine, promethazine, chlorpromazine, etc., during trial medication period.

? Withdrawal of consent by patient/parent/guardian.

? If the patient is lost to follow up

Assessment of efficacy

Primary efficacy end-points

? Cumulative cure rate by Day 14 for chloroquine and Day 28 in case of SP (for definition see ACR)

Treatment failure is defined by the occurrence of any of the following:

Early Treatment Failure (ETF):

? Development of danger signs or severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitaemia.

? Axillary temperature $\geq 37.5^{0}$C on Day 3 in the presence of parasitaemia.

? Parasitaemia on Day 3 $\geq$ 25% day 0 count.

Late Treatment Failure (LTF):

? Development of danger signs or severe malaria on any day from Day 4 to Day 14:

? Axillary temperature $\geq 37.5^{0}$C in the presence of parasitaemia on any day from Day 4 to Day 14 without previously meeting the criteria of early treatment failure.

Success Criteria:

? Cumulative cure rate by Day 14 for chloroquine and Day 28 in case of SP.

Adequate Clinical Response (ACR):

? Absence of parasitaemia on Day 14 irrespective of axillary temperature, without previously meeting any of the criteria of early treatment failure;

? Axillary temperature $\geq 37.5^{0}$C irrespective of the presence of parasitaemia, without previously meeting any of the criteria of early or late treatment failure.

Secondary efficacy end-points

? Parasite clearance time (PCT: days): defined as time between instituting therapy and clearance of (asexual *P. falciparum*) from peripheral blood film detectable by microscopy and remain cleared of asexual parasites (negative) through out the follow up period.

? Proportion of patients with parasitaemia (asexual *P. falciparum*) by 24 hours (Day 1), and 72 hours (Day 3).

? Fever clearance time (FCT: days): defined as time required for axillary temperature to fall below 37.5^{0}C and remain so for at least 72 hours.

- Proportion of afebrile patients (axillary temperature less than 37.5 ^{o} C) by 24 hours (Day 1), 48 hours (Day 2) and 72 hours (Day 3)
- Proportion of patients with gametocytes on days 7, 14, 21 and 28, stratified by those with and without gametocytes on day 0.
- Haematological response: Haemoglobin/haematocrit on day 14 (for CQ) and 28 for SP.

Data analysis

The analysis of the efficacy data should be performed for the *"Intention-To-Treat"* (ITT) patient population, i.e., all recruited patients, and the *"Evaluable Patient Population" or "Per-Protocol Population (PP)"*. All patients properly recruited (all inclusion/exclusion criteria met) to any treatment group should qualify for the ITT analysis. The ITT analysis should be applied for all parameters and also include patients who discontinue before completion of follow-up due to withdrawal of trial medication, discontinuation (end-points reached), and lost to follow-up. Patients will be eligible for PP efficacy analysis provided the criteria in the ITT population has been satisfied and the following apply: (i) The patient has completed the course of antimalarial therapy and follow-up, (ii) No major protocol violation exists with regard to inclusion/exclusion criteria, and (iii) No prohibited concomitant medications were taken during the follow-up period.

Case Report Form

All clinical data will be recorded in the *Patient Record File (PRF)*, from where transcription will be made into the *"Case Report Form" (CRF).* Original laboratory data will be attached to the PRF and only the results will be transferred into CRF. The PRF will in addition contain the names of the patient's mother &/or Father and a clear description of how to reach the home of the patient for use in the event of patient default.

The CRF should contain essential information (minimal data) as follows:

Screening

- ***Informed consent***
- ***General Information***
  - Date of screening
  - Patient initials
  - Patient hospital number
  - Study ID
  - Sex: M/F

- Date of birth (dd/mm/yy)
- Age (months)
- Weight (kg)
- Height (cm)
- No of attacks of malaria within the preceding 6 months
- History of previous antimalarial therapy in the preceding 4 weeks Y/N
- Significant Past medical history
- Contact address (brief description of how to reach patient's home)

? ***Presenting complaints***

- Fever
- Nausea
- Vomiting
- Diarrhoea
- Haematuria
- Cough
- Dyspnoea
- Seizure
-

? ***Inclusion/exclusion criteria*** *(see text)*

? ***Pre-medication assessments***

Full physical examination

General:

Pallor, jaundice, axillary temperature, skin rash, oral candidiasis, oedema, mucous membranes, skin tugor, fontanel and state of hydration.

Nutritional status: flaky-paint skin, fluffy (silky) discoloured hair, visible wasting.

Cardiovascular system: heart rate, blood pressure, gallop rhythm and heart sounds.

Respiratory System: nasal flaring, in drawing of the intersoastals, deep breathing, respiratory rate, crackles, crepitations, bronchial breathing, wheeze.

Abdomen: *hepatomegaly, splenomegaly – characteristic of liver and splenic enlargement.*

ENT Examination*:* visible pus in ear, inflamed ear drum, tonsillitis, and pharyngitis.

Neurological: *Level of consciousness (Blantyre coma score), neck stiffness, eye movements, verbal response, motor response, convulsion, ability to sit or stand unsupported.*

? Peripheral blood parasitaemia

? Haematocrit/haemoglobin

? Random blood glucose

During the Study

? Antimalarial drug administration – all doses of antimalarial drug to be administered supervised

? Assessments during follow-up

-Physical examination

-Axillary temperature

-Peripheral blood parasitaemia

-Haematocrit/haemoglobin

- Concomitant medication
- Non-pharmacological measures
- Rescue therapy case of treatment failure

Study Termination

- Complete study termination page and fill in final assessment of efficacy.

Measures to minimise or avoid bias

- For clinical trials with the objective of comparing the clinical efficacy of several antimalarial drugs or regimens, patients who meet all inclusion criteria should be randomly allocated to receive study medications. The principal investigator prior to study initiation will generate the randomisation list.

Procedures for monitoring patient compliance

- The physician or nurse will administer all study drugs, and confirm ingestion by inspection of the mouth.
- Study participants should be asked to refrain from taking drugs other than those prescribed by the study team. Every study participant should be given an identifier card to take home with him/her. The card will contain information for other health care providers to consult the study team in case the study participant presents to their health care facility with any ailment during the follow-up period. If for any reason the participant receives treatment outside the study team, this should be recorded on the PRF and CRF indicating the name of the medication, dosage, and date of administration. The study physician will evaluate the concomitant drug in relation to the study and take a decision on the need to continue with the study or withdraw the patient.

Quality assurance

- Quality assurance and quality control systems with written *"Standard Operating Procedures*" (SOPs) should be implemented to ensure that the studies are

conducted and data are generated, recorded and reported in compliance with the protocol, and GCP.

- A centralised supply of drugs and laboratory equipment and supplies is preferable to ensure standardisation and good quality of the material.
- A central laboratory will generate a random sample of slides (10%) and related records, which will be forwarded to the regional or central laboratory or an official reference laboratory at regular intervals.
- MIM/TDR as the sponsoring agency will perform monitoring visits, which will include all source data verification and inspection of CRFs. In addition, the adequacy of the clinical and analytical facilities/methodologies will be assessed.

Ethical considerations

The clinical assessment of antimalarial drug resistance should be conducted in accordance with the principles laid down by the World Health Assembly of 1975 on Ethics in Human Experimentation and the Helsinki Declaration. The study will adhere to the standards established for Good Clinical Practices (GCP) and conform to the TDR Standard Operating Procedures (SOP). Prior to study initiation, approval of the study protocol from the local IEC/IRB and the WHO Secretariat Committee for Research Involving Human Subjects (SCRIHS) is required.

Written informed consent: Written or witnessed verbal informed consent must be obtained from each prospective patient volunteer, the parent or legal guardian at the start of the study. Each subject, parent or guardian will be informed of the objectives, methods, anticipated benefits and potential hazards of the study.

The subject, parent or guardian must be informed that he/she is at liberty to abstain from participation in the study and that he/she is free to withdraw the consent of participation at any time without loosing any benefits which the parent/guardian or child are entitled.

Requirements for the *in vivo* test

Clinical:

- First line antimalarial drug
  - Chloroquine, amodiaquine, sulfadoxine / pyrimethamine [SP]
- Rescue antimalarial drugs
  - Mefloquine, halofantrine, artemether or SP

- Alternative drugs of first-line drug for severe or complicated malaria (for patients who deteriorate)
  - Parenteral quinine and artemether, suppository formulation of the artemisinin derivatives.
- Ancillary drugs
  - Antipyretic analgesics e.g. paracetamol,
  - Antibiotics e.g. amoxicillin
  - Iron preparation according to site-specific practice in children who are anaemic.
- Clinical supplies
  - Diagnostic set (laryngoscope, ophthalmoscope, pen touch) + spare batteries, disposable spatula/tongue depressor, disposable syringes and needles, digital infant weighing scale spare batteries, glucometer and glucometer strips, electronic thermometers, permanent markers, plastic cups and biscuits, light microscope, lens tissue, xylene (to clean microscope lens), heparinized capillary tubes, placticine – to seal capillary tubes, slide trays and slide racks.
- General/Stationary supplies
  - Clinic note books – attendance register, screening register, follow up notebook.
  - PRF, CRF,
  - Patient cards
  - Laboratory forms
  - Ball pen – black/blue/red.

Laboratory:

- Light microscope, microscope slides with frosted edge, lens tissue, haemolancet, heparinised haematocrit tubes, haemofuge, rubber gloves, immersion oil, xylene, slide box, methanol/box, swabs / alcohol, Giemsa stain stock solution , distilled water, Isocode Stix, capillary tubes, cryotubes (1.5 mL) for blood sample collection, disposable pipettes, count down timers, buffer tablets pH 7.2.

Table 1 Time and events schedule

Assessment	Chloroquine /Amodiaquine Treatment				Follow-up through Day 28					At Recurrence
	S/P treatment									
	Day 0 Screening/ Baseline	Day 1	Day 2	Day 3	Day 4 –6	Day 7	Day 14	Day 21	Day 28	
Written Informed Consent	✍									
Inclusion/Exclusion Criteria	✍									
Demography	✍									
Medical History	✍									
Clinical Examination (Including PE, vital signs, axillary temperature)	✍	✍	✍	✍	✍	✍	✍	✍	✍	✍
Assessment of Clinical Symptoms of Malaria	✍	✍	✍	✍	✍	✍	✍	✍	✍	✍
Concurrent Medication Review	✍	✍	✍	✍	✍	✍	✍	✍	✍	✍
Malaria Finger-prick blood smears	✍	✍		✍	✍	✍	✍	✍	✍	✍
Haemoglobin/Haematocrit	✍					✍			✍	
Blood glucose	✍									
[a] Blood Sample for Parasite DNA (filter paper)	✍					✍	✍	✍	✍	✍
[a]Blood Sample and *in vitro* Susceptibility Testing	✍									✍
Blood for drug levels	✍[b]	✍[c]		✍[c]		✍[c]	✍[c]	✍[c]	✍[c]	✍[c]

a- finger prick blood
b-1.5 ml blood for baseline concentrations of chloroquine / n-desethylchloroquine, amodiaquine / n-desethylamodiaquine and sulphadoxine / pyrimethamine
c- 0.5 ml blood each, for determination of chloroquine/n -desethylchloroquine, or amodiaquine / n-desethylamodiaquine or sulphadoxine / pyrimethamine concentrations in whole blood